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ATTORNEY'S DOCKET NUMBER: P23,565-A USA

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5): 09/869685

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/31284	30 December 1999 (30.12.99)	30 December 1998 (30.12.98)

TITLE OF INVENTION: **PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS**

APPLICANT(S) FOR DO/EO/US: **Rene Bruno**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau)
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C 371(c)(2))
   a. ☐ is attached hereto
   b. ☐ has been previously submitted under 35 U.S.C 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a ☐ are attached hereto (required only if not communicated by the International Bureau)
   b. ☐ have been communicated by the International Bureau
   c. ☐ have not been made, however, the time limit for making such amendments has NOT expired
   d. ☒ have not been made and will not be made
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C 371(c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C 371 (c)(4)) (executed).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C 371 (c)(5))
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409), mailed 12 April 2001 (12.04.01).
12. ☒ A copy of the International Search Report (PCT/ISA/210), mailed 29 June 2000 (29.06.00).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1 97 and 1 98
14. ☒ An assignment document for recording A separate cover sheet in compliance with 37 CFR 3.28 and 3 31 is included
15. ☐ A **FIRST** preliminary amendment
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
17. ☐ A substitute specification
18. ☐ A change of power of attorney and/or address letter
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1 821 - 1 825
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4)
21. ☐ A second copy of the English language translation of the international application under 35 U.S C 154(d)(4)
22. ☒ Certificate of Mailing by Express Mail, dated 29 June 2001 (29.06.01).
23. ☐ Other items or information

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/869685	PCT/US99/31284	P23,565-A USA

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1000.00	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	29 - 20 =	9	x $18.00	$162.00
Independent claims	3 - 3 =	0	x $80.00	$0.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
	TOTAL OF ABOVE CALCULATIONS		=	$1,022.00
☐	Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.			$0.00
	SUBTOTAL		=	$1,022.00
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).		+	$0.00
	TOTAL NATIONAL FEE		=	$1,022.00
	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☒			$40.00
	TOTAL FEES ENCLOSED		=	$1,062.00
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$1,062.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **19-5425** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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34,638
REGISTRATION NUMBER

29 June 2001 (29.06.01)
DATE

PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS

Field of the Invention

This invention relates to methods which are useful in the treatment of cancer with a pharmaceutical that is a member of the family of anti-neoplastic agents known as taxoids.

The family of anti-neoplastic agents known as taxoids are based on natural and modified compounds that have the taxone skeleton isolated from the yew tree (Taxaceae). Two particularly effective taxoids are paclitaxel, which is a natural product isolated from the Pacific yew (Taxus brevifolia), and docetaxel, which is a semisynthetic product derived from the needles of the European yew (Taxus baccata). The activities of these agents have been demonstrated in a wide variety of cancers, including breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. Other taxoids are being developed for cancer treatment also.

The taxoids appear to show a common mechanism of action based on promoting the assembly and inhibiting the disassembly of microtubules. This causes disruption of the microtubular network that is required for mitotic and interphase cellular functions thereby disrupting cell proliferation.

For a patient being treated with a taxoid, it is desirable to be able to predict the efficacy of the treatment and/or a suitable dosage level to administer to the patient. Efficacy can be characterized by the response to taxoid treatment and the survival of the patient. Suitable dosage levels relate to reducing or avoiding undesirable side effects that might be experienced by the patient while maintaining efficacy.

Reported Developments

Descriptions of clinical studies relating to the efficacy and side effects resulting from the clinically available taxoids, paclitaxel and docetaxel (TAXOTERE®) are provided in The Physicians Desk Reference, 52nd edition (1998) p762-766 (paclitaxel) and 2385-2389(TAXOTERE®). An extensive review of the clinical and preclinical profiles of docetaxel are presented in Cortes, J.E., and Pazdur, R.J., Clin. Oncol. 13(10) 2643-2655,(1995). An extensive review of the chemotherapy trials treating advanced breast cancer using the taxoids is provided in Clemens, M. et al., Eur. J. Cancer 33(13) 2183-21939(1997). A review of the pharmacokinetic parameters of paclitaxel and docetaxel and side effects experienced with the use of these taxoids is provided in Verweij, J. et al Ann. Oncol 5(6) p495-503 (1994).

Summary of the Invention

In accordance with the present invention, there is provided a method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein (AAG). The method includes observing the patient's level of AAG,

evaluating the AAG level to determine the dosage of a taxoid to administer to the patient by comparing the AAG level to a predetermined AAG level from a population of patients that have the same type of cancer and who are being treated with the taxoid at a common dosage, and, based on this evaluation, recommending the dosage of the taxoid to administer to the patient.

The taxoids can be, for example, docetaxel or paclitaxel. Examples of the type of cancers that can be treated include breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. An example of a preferred of a preferred embodiment of the present invention involves the treatment of non-small cell lung cancer.

Another aspect of the present invention is the provision of a method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid. This method includes observing the patient`s AAG level, comparing the AAG level to a predetermined AAG level derived from a population of patients having the same cancer and being treated with the taxoid at a common dosage, and, based on this comparison, assessing the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient.

In preferred embodiments, the patient is treated with a dosage of about 55 mg/m^2 to about 200mg/m^2 of the taxoid. In especially preferred embodiments of the invention, the patient is treated with about 55 to about 125 mg/m^2 of docetaxel or about 135 to about 175 mg/m^2 of paclitaxel.

Still another aspect of the present invention is the provision of a method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid. This method includes observing the patient's AAG level, comparing the AAG level to a predetermined AAG level derived from a population of patients

having the same cancer and treated with the taxoid at a common dosage, and, based on this comparison, recommending the dosage of the taxoid to administer to the patient to reduce the incidence or severity of side effects that the patient may experience during treatment with the taxoid.

Examples of side effects include neutropenia, infection, diarrhea, infusion related hypersensitivity reactions, allopenia, neurotoxicity, mucositis, stomatitis, sever asthenia and myalgia. Neutropenias include febrile neutropenia.

Brief Description of the Drawings

Figure 1 is a pharmacokinetic profile of the taxoid docetaxel in a representative patient with normal liver function (□) and a patient with elevated hepatic enzymes (■). Lines denote model predictions after Bayesian estimation.

Figure 2 is a docetaxel pharmacokinetic profile in a subset of 254 patients.

Figure 3 is a model-predicted probability of febrile neutropenia as a function of CLf for a patient with median AAG. Reference vertical lines denote normal CL (CLf=1) and 50% reduced CL (CLf=2).

Figure 4 shows survival curves in NSCLC patients with low (≤1.11 g/L, --), intermediate (1.12 to 1.84 g/L,……), and high (≥1.85,-·-·-) base line AAG (/censored observation).

Detailed Description of the Invention

In connection with the development of this invention, it has been found that, for a cancer patient being treated with a taxoid, the patient's level of alpha-1-acid glycoprotein can be used to predict response to treatment, survivability, and side effects.

For background purposes there is set forth hereafter information relating to the taxoids and alpha-1-acid

glycoprotein(AAG). Following this information, methods for measuring AAG levels are described and the relationships between AAG levels and response to treatment, survivability, and side effects are discussed. With regard to side effects, methods for reducing the possibility of side effects by measuring a patient's AAG level prior to or during taxoid treatment and adjusting the dosage of the taxoid are discussed.

Taxoids

The present invention relates to treatment methods utilizing taxoids. A variety of taxoids may be used in the practice of the present invention. "Taxoid" as used herein refers to anti-neoplastic agents based on natural and modified compounds that have the taxane skeleton isolated from the yew tree. Preferred taxoids used in the practice of the invention are paclitaxel and docetaxel. Paclitaxel promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. Docetaxel binds free tubulin and promotes assembly of microtubules while simultaneously inhibiting the disassembly of the microtubules. This results in the stabilization of microtubules and inhibition of mitosis. The use of docetaxel is particularly preferred in the practice of the invention.

Paclitaxel has the chemical formula $C_{47}H_{51}NO_{14}$ and has a molecular weight of 853.9. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine (*Physician's Desk Reference, supra*)).

Docetaxel has the chemical formula $C_{43}H_{53}NO_{14}\cdot 3H_2O$ and has a molecular weight of 861.9. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-phenylisoserine,N-*tert*-butyl ester,13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate (see *Physician's Desk Reference, supra*).

In the practice of the present invention, any cancer that responds to treatment with the taxoids may be treated using the methods of the present invention. The taxoids are known to have activity against a variety of cancers, including, for example, breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. The taxoids have demonstrated activity against other types of cancers when used alone or in conjunction with other anti-neoplastic agents. The present invention is particularly well suited to patients undergoing treatment for non-small cell lung cancer (NSCLC).

Alpha-1-Acid Glycoprotein

There follows hereafter a description of the biochemical and genetic characteristics of alpha-1-acid glycoprotein (AAG) and a description of its function in the body. This is followed by a description of methods for measuring AAG levels and a discussion of the significance of AAG levels found in patients treated with a taxoid.

AAG Characteristics and Function

Alpha-1-acid glycoprotein (AAG), also called orosomucoid, is found in the seromucoid fraction of human blood plasma. Northern blot analysis of RNA extracted from a variety of tissues demonstrates preferential expression of AAG in the liver. The complete amino acid sequence of AAG is known (Schmid, K. et al., *Biochemistry*, 12, 2711-2724 (1973)) and the carbohydrate moiety has been also identified (Schmid, K. et al., *Prog. Clin. Biol. Res.*, 300, 7-2 (1989)). AAG consists of a single polypeptide chain of 183 amino acids with 21 substitutions possible having a molecular weight of approximately 21 kDa. Within the protein backbone there are five N-glycosylation sites for the attachment of oligosaccharides. There are believed to be at least seven alleles coding for AAG (Yuasa, I. et al., *Hum. Genet.*, 77, 255-258 (1987); Umetsu, K. et al., *Electrophoresis*, 9, 224-226 (1988)). Three phenotypes have been identified with autosomal co-dominant transmission. The variant forms result from amino acid substitutions. The structure and expression

of the genes coding for AAG have been described and the AAG locus has been mapped to the distal portion of the long arm of chromosome 9 (Dente, L. et al., Embo J., 6, 2289-2296 (1987); Eiberg, H. et al, *Clin. Genet.*, 23, 150-154 (1983)).

The normal function of AAG in the body is not completely understood. Based on *in vitro* studies, AAG may be involved in coagulation, phagocytosis, graft rejection, and wound healing. A review of the biological activities of AAG may be found in Kremer et al.(*Pharm. Rev.*, 40(1), 1-47 (1988)).

AAG is classified as an "acute phase protein" because the concentration of AAG increases following inflammatory stimuli. AAG synthesis also increases several fold during an acute phase response (Ricca et al., *J. Biol. Chem.*, 256, 11199-11202 (1981); Koj et al., *Biochem J.*, 206, 545-553 (1982); Koj et al., *Biochem J.*, 224, 505-514 (1984)). The major inducers of AAG synthesis are the cytokines interleukin-1 (IL-1) and interleukin-6 (IL-6), which act additively to induce transcription of the AAG gene.

Increased levels of AAG due to the acute response have been identified in cancer patients and it has been found that elevated levels of AAG can effect the efficacy of a variety of drugs.

AAG has long been known to bind drugs in plasma. AAG demonstrates high binding affinity for basic drugs (with pK values of 8 or higher). In addition, acidic and neutral drugs have also been shown to bind AAG. For an extensive review of the drug binding activity of AAG, see Kremer et al., (*Pharm. Rev.*, 40(1), 1-47 (1988)).

It is known also that the taxoids are bound by AAG. *In vitro* studies have demonstrated that up to 98% of the taxoid docetaxel is bound by plasma proteins, including AAG (see *Physician's Desk Reference, supra*). Similarly, *in vitro* studies of the binding of paclitaxel to serum proteins at paclitaxel concentrations from 0.1 to 50 μg/ml indicate that

89 to 98% of the paclitaxel is bound. Accordingly, it is expected that AAG has the ability to bind taxoids *in vivo*. Due to this binding interaction, the level of AAG in a patient's plasma becomes significant because, as more AAG is available, a greater percentage of an administered taxoid may be bound.

The present invention involves the relationship between AAG levels in a patient who is treated with a taxoid and response to treatment, survival, and side effects. Accordingly, the present invention involves the measurement of a patient's AAG level.

Methods for Measuring AAG Levels

A variety of bodily fluids and tissues may be used to evaluate a patient's AAG level in connection with the practice of the present invention. Methods of obtaining samples of bodily fluids and tissues are well known in the art. Blood plasma is the preferred bodily fluid used to determine AAG levels in the practice of the present invention. Guidance on obtaining blood samples in order to determine AAG levels may be found in Bienvenu et al., *Clinical Chemistry*, Vol. 27, No. 5, 1981, and in Ganz et al., *JNCI*, Vol. 71, No. 1, July 1983. In preferred embodiments utilizing blood plasma to determine AAG levels, a suitable volume of blood, for example, about 0.5 to about 0.2 ml is taken from a patient, preferably by venipuncture, and is centrifuged under sufficient conditions to isolate the plasma fraction for analysis of the AAG level.

A variety of methods known in the art can be used to determine a patient's AAG level. These methods include methods known for isolation and quantification of a protein. Suitable techniques for isolating and quantifying a protein may be found in a variety of sources, including e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, volumes I and II

(D.N. Glover ed. 1985); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994). Methods used to quantify AAG preferably utilize antibodies. A general overview of immunoassays is provided in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Pubs., NY (1988). Monoclonal or polyclonal antibodies specific for AAG can be used in immunoassays to quantify AAG. Monoclonal antibodies against AAG may be obtained from commercial sources, such as ICN Pharmaceuticals Inc. (Catalog No. 692011) or prepared using techniques known in the art. To produce monoclonal antibodies that bind AAG, hybridomas producing anti-AAG antibodies can be prepared and selected for as described in the literature. For example, mice (i.e., balb/c mice) can be immunized with AAG by intraperitoneal injection. After sufficient time has passed to allow for an immune response, the mice can be sacrificed and the spleen cells obtained and fused with myeloma cells using techniques well known in the art. The resulting hybridomas are then grown in a selective medium, and the surviving cells grown in such medium using limiting dilution conditions. After cloning and recloning, hybridomas can be isolated that secrete antibodies (for example, of the IgG or IgM class) directed against AAG. Immunoassays which can be used to quantitate AAG may include ELISA, competitive immunoassays, radioimmunoassays, indirect immunofluorescent assays and the like. Preferred methods for quantification of AAG include, but are not limited to rocket immunoabsorbant assays (Dewey et al., *J. Immunol.*, 144, 4392-8 (1990); radioimmunoassays (Ganz et al., *JNCI*, 71(1), 25 (July 1983); laser nephelometry (Bienvenu et al., *Clinical Chemistry*, 27(5), 721-726 (1981); and immunoassay in a Cobas Bio centrifugal analyzer (Verme et al., *Clinical Chemistry*, 34(1), 2316-2320 (1988)).

A highly preferred method for determining a patient's AAG level utilizes laser nephelometry as described in Bienvenu et al., *Clinical Chemistry*, 27(5), 721-726 (1981). In this method, a blood sample (0.4 ml to 0.2 ml) is collected by venous puncture, and the serum is removed after

centrifugation. A Behring Laser Nephelometer module I (Behringwerke, D-3550 Marburg/Lahn, Germany) is utilized for taking measurements. Samples, standards, and antisera are diluted with sterile isotonic saline solution and 100 μL of 101-fold diluted sample is mixed in a microcuvette with 200 μL of a fivefold diluted anti-orosomucoid antiserum (LN serum anti-orosomucoid (AAG) SAW; Behringwerke (or other commercially available or prepared antibody)). The cuvettes are shaken briefly and allowed to stand for 1 hour at room temperature, and the light scattered by the resulting antigen-antibody complexes is measured (in volts) with the nephelometer. A calibration curve is prepared by use of an 800mg/L standard solution of AAG, diluted to give concentrations of 40, 20, 10, 5, 2.5, and 1.25 mg/L and the concentration of the AAG in the sample is calculated based on its light scattering relative to the known standards.

The present invention includes within its scope situations in which a patient's AAG level is determined by a third party not involved in the predictive aspects of the present invention.

Significance of AAG Levels

The present invention is based in part on the discovery that, for cancer patients being treated with a taxoid, the patient's AAG level is a prognostic factor which allows predictions to be made regarding response to treatment, survival, and side effects. An explanation follows respecting the significance of AAG levels which are higher or lower than the norm or that fluctuate during treatment.

Typically blood or other body fluid samples are taken after a cancer has been diagnosed and during taxoid treatment. Samples may be taken at any point prior to or during the course of treatment. Blood samples obtained prior to manifestation of cancer may be useful in determination of a baseline AAG level in the absence of disease.

The range of AAG in normal individuals is from about 0.36 g/L to about 1.46 g/L. The level of AAG is often elevated in pathological states such as liver cirrhosis, renal disease and cancer. Individuals with cancer may have elevated AAG levels. For example, a study of patients (n=180) having NSCLC had levels of AAG ranging from about 0.84 to about 2.71 g/L, with a median of 1.42 g/L. Approximately half of the patients had an AAG level exceeding the maximum AAG level (1.46 g/L) seen in healthy subjects.

Methods known in the art can be used to evaluate and classify ranges of AAG concentrations in "taxoid-treated" patients with various types of cancer. A sample population of patients having a particular type of cancer who are being treated with a particular taxoid at a common dosage may be studied to quantify the relationship between AAG levels and response to treatment, survival and side effects. The term common dosage refers to a population all of which are receiving the same dosage of a taxoid, for example, a dosage of about 100 mg/m^2. Given a common dosage, patients within a population may receive differing absolute amounts of a taxoid depending on their size. The dosage of a taxoid being administered to a population will depend on the type of cancer and the taxoid being used. Generally speaking, it is believed that the taxoid dosage will fall within the range of about 55 to about 200 mg/m^2, but may be higher or lower, as conditions warrant. A typical dosage range will usually be about 75 mg/m^2 to about 175 mg/m^2. For a given type of cancer, the range of AAG concentrations in the population may be obtained and defined as high, intermediate, and low, using data from the population of patients and standard statistical methods. The AAG concentration ranges in the population constitute "predetermined" AAG levels which are then used for comparison and evaluation of an individual patient's AAG level. For example, for a population of patients, the 25% quantile of the AAG distribution in the population can be classified as the low level and the 75% quantile can be classified as the high level, with the >25% quantile to <75% quantile being classified as intermediate level.

Accordingly, in the practice of the present invention, the AAG concentration is determined using any of the assays described above, preferably the Bienvenu et al. Laser Nephelometry assay. Once the AAG level is known, the patient may be classified as having a high, intermediate, or low AAG level according to the quantile level into which the patient's AAG level falls. After the patient's AAG level has been classified, this level may be compared with observations on the relationship between AAG level and response to treatment, survival, and side effects for a patient population having the same type of cancer and being treated with the same type of taxoid.

Response to Treatment

The term "response to treatment" refers to whether a patient responds to treatment according to the standard criteria for partial response (PR=50% reduction in tumor) and complete response (CR= a complete reduction in tumor) as defined by the National Cancer Institute. Non-response is defined as patients with minor responses (< 50 % reduction in tumor size) evaluable disease, stable disease and patients with disease progression.

The present invention provides methods for assessing the effect of treatment of a given cancer with a given taxoid based on observing the patient's AAG level, comparing this level to a predetermined AAG level derived from a population of patients having the same type of cancer and being treated with the same taxoid at a common dosage, and assessing the effect of continued treatment.

The chances of a patient responding to treatment with a taxoid relates to the patient's AAG level. It has been found that there is a significant increase in the chance of a patient responding to taxoid treatment for a patient who exhibits a low AAG level. In general, if a patient has a low AAG level there is an increased chance of response to taxoid treatment relative to the chance of response for a patient with high AAG levels.

Accordingly, a blood sample can be obtained from a patient and the observed AAG level classified as high or low according to the guidance provided hereinabove. The AAG level is evaluated in order to consider recommending an adjustment in the taxoid dosage and/or supplementing treatment with additional chemotherapeutic, surgical or radiation treatments to increase the chance of response to treatment. Based on the AAG level, the patient can be classified as having an increased chance of response if the patient has a low AAG concentration. For a patient with a high AAG level, the patient's response may be considered to be reduced relative to patients having low AAG concentrations.

If a quantitative characterization of the relationship between AAG and response rate is desired for a particular type of cancer, one of skill in the art can readily obtain blood samples from a population of patients having a given type of cancer and follow the guidance provided in the Examples below to further define the correlation between AAG levels and response to treatment with a taxoid.

Survival

The term "survival" is defined as the length of the patient's life from the time of the first infusion of a taxoid dosage to the date of death. The present invention provides methods of assessing the effect of treatment as it relates to survival for a patient who has cancer and who is being treated with a taxoid. The method involves observing an individual patient's AAG level, classifying the AAG level as low, intermediate or high AAG compared to predetermined AAG levels in a patient population having the same type of cancer under treatment with the same taxoid at a common dosage and assessing the effect of continued treatment in order to predict the patient's survival. The AAG level is evaluated in view of a population of patients having the same type of cancer to consider recommending an adjustment in the taxoid dosage and/or supplementing the treatment with additional chemotherapeutic, surgical or radiation treatment

to prolong survival. In accordance with the present invention, it has been found that patients having low AAG levels will be expected to survive longer than patients with high AAG levels.

If a quantitative description of the relationship between AAG level and survival is desired for a particular type of cancer, one of skill in the art may obtain blood samples from a population of patients having that type of cancer and being treated with the same taxoid at a common dosage and may follow the protocol presented in the Examples below to further define the relationship between AAG level and survival.

In addition to survival, the methods of the present invention are also useful in predicting time to progression. Time to progression is calculated from the first administration of the taxoid to the date of progression as discussed in the examples below. Studies of patients with NSCLC demonstrated that patients with low AAG levels ($\leq$ 1.09 g/L) had a longer time to progression (18 weeks) versus 9.7 weeks for patients with high AAG levels ($\geq$ 1.92 g/L). Accordingly, the methods described above for survival may also be used with regard to time to progression.

Side Effects

The term "side effects" refers to adverse effects produced by a drug such as a taxoid, especially on a tissue or organ system other than the one sought to be treated with the drug. Use of the taxoids can result in a variety of side effects, including, for example, neutropenia, infusion-related hypersensitivity reactions, allopecia, neurotoxicity, mucositis, infections, stomatitis, diarrhea, severe asthenia, fluid retention and myalgias.

The nature and severity of the side effects due to the use of a given taxoid will depend on a variety of factors, including the specific taxoid used, the dosage, the overall

dosing regimen, the presence of other drugs, and factors relating to the patient's physiological state.

The side effects specific to paclitaxel and taxotere are well documented (see *Physician's Desk Reference, supra*, Cortes and Pazdur, *Journal of Clinical Oncology*, vol. 13, No. 10, 2643-2655 (October 1995)). The major dose-limiting side effect of paclitaxel is neutropenia. Other side effects include dose dependent mucositis and peripheral neuropathy, cardiac rhythm abnormalities, arthralgias/myalgias, hypersensitivity reactions, alopecia, nausea and vomiting. The major dose limiting side effect of docetaxel is neutropenia. Other side effects include paresthesias, hypersensitivity reactions, alopecia, skin reactions, fluid retention, nausea, vomiting and diarrhea. A discussion of the side effects experienced with paclitaxel and docetaxel (TAXOTERE®), may be found in the *Physician's Desk Reference, supra*. These side effects may be defined and graded using the common toxicity criteria of the U.S. National Cancer Institute or COSTART classification. The patient's AAG level may be used to predict the possibility of a variety of side effects, in particular, grade 4 neutropenia, infection and grade 3 diarrhea.

The present invention provides methods for assessing the effect of treatment as it relates to side effects for a patient who has cancer and who is being treated with a taxoid. The method involves observing the patient's AAG level, classifying the AAG level as high, intermediate or low compared to predetermined AAG levels derived from a population of patients having the same type of cancer and being treated with the same taxoid at a common dosage, and based on this comparison assessing the side effects that may be experienced by the patient.

The present invention also provides a method for assessing whether a patient who has cancer and who is to be treated with a taxoid will experience side effects. The method involves observing the patient's AAG level prior to

treatment and comparing this level to a predetermined AAG level derived from a population of patients having the same type of cancer and being treated with the same taxoid that is to be used in treating the patient, and, based on this comparison recommending a dosage of the taxoid that will reduce or eliminate side effects that may be experienced by the patient, while providing an improvement or cure in the patient's condition.

The odds of experiencing side effects resulting from taxoid treatment can be predicted based on AAG levels. The relationship between AAG levels and the occurrence of side effects is believed to relate to the AAG-taxoid binding interaction. In accordance with the present invention, it has been found that patients with high AAG levels are less likely to experience adverse side effects than patients with low AAG levels.

If a quantitative characterization of the relationship between AAG and side effects is desired for a particular type of cancer, one of skill in the art can readily obtain blood samples from a population of patients having a given type of cancer and follow the guidance provided in the Examples below to further define the relationship between AAG levels and side effect(s) due to treatment with a given taxoid.

Determination of Dosage Levels

Based on the ability to predict response to treatment, survival, and side effects, the present invention may be used to adjust the dosage of a taxoid being administered to a patient. Accordingly, the present invention provides methods for determining the dosage of a taxoid to administer to a patient being treated for a cancer. These methods involve observing the patient's level of AAG and evaluating the AAG level to determine the dosage of the taxoid to administer to the patient by comparing the patient's AAG level to a predetermined AAG level in a population of patients who have the same type of cancer and who are being treated with the same taxoid at a common dosage. Based on this information, a

recommendation can be made on the dosage of taxoid to give to the patient. With regard to response rate, a patient who has a high AAG level and who is predicted to have a decreased chance of responding to treatment may have their taxoid dosage increased. Similarly, a patient who has a high AAG level and who is predicted to have a reduced length of survival may have their taxoid dosage increased. Given that patients with high levels of AAG are also less likely to experience side effects, it may be possible to increase the AAG dosage for these patients without a corresponding increase in the possibility of side effects.

Given the correlation between AAG levels and side effects, one method for reducing the possibility of side effects is to determine the patient's AAG level and to adjust the taxoid dose so as to reduce the possibility of side effects.

Accordingly, the present invention also provides methods for reducing the chance of a cancer patient's experiencing side effects from a taxoid by observing the patient's level of AAG prior to treatment and classifying the patient's level of AAG as high or low. If the patient has a low AAG level, the dose of a taxoid to be administered to the patient may be reduced so as to reduce or eliminate any possible side effects.

The dosage levels for taxoids are specific to the particular taxoid being used and the cancer being treated. Dosage recommendations for the clinically available taxoids are provided with the products and may also be found in the Physicians' Desk Reference and in the scientific literature. Dosage recommendations for the taxoid docetaxel range from about 55 mg/m^2 to about 125 mg/m^2. These dosages are usually administered intravenously over 1 hour every three weeks. Dosage recommendations for the taxoid paclitaxel range from about 135 mg/m^2 to about 175 mg/m^2. These dosages are usually administered intravenously over 3 hours every three weeks.

Guidance for adjusting taxoid dosage based on actual side effects may be found in the *Physician's Desk Reference*, 52nd ed., (1998) (for TAXOL® (paclitaxel) see p762-766 and for TAXOTERE® (docetaxel) see p2385-2389). These recommended adjustments based on actual side effects may be used as a guide to adjusting dosage based on predicted side effects.

With regard to paclitaxel, patients who have low AAG levels and would be predicted to experience neutropenia or other side effects such as infection or grade 3 diarrhea during paclitaxel therapy may have their paclitaxel dosages reduced by about 5 to about 35%, preferably by about 10 to about 30%, even more preferably from about 15 to about 27%.

With regard to docetaxel (Taxotere®), patients who would be predicted to experience neutropenia, including febrile neutropenia or other side effects may have their docetaxel dosage reduced by about 5 to about 35%, preferably by about 10 to about 30%, even more preferably about 15 to about 27%. If the side effects actually occur, the dosage may be further decreased.

In general, the taxoid dosage may be adjusted upwardly, or downwardly, based on actual side effects and response to treatment.

Description of an Embodiment of the Invention

The methods of the present invention are illustrated in the Examples below which describe a study involving NSCLC cancer patients who were treated with the taxoid docetaxel (Taxotere®). The study involved a determination of the relationship between AAG levels and response to treatment, survival, and side effects.

The study included 180 NSCLC patients who were enrolled in six Phase II studies of 100 mg/m^2 of docetaxel.

The AAG levels of the patients were determined and classified into high (≥1.85 g/L (75 percentile and above)),

intermediate (1.12 to 1.84 g/L (26 percentile to 74 percentile), and low (≤1.11 g/L (25 percentile and below) levels.

The general relationship between AAG levels and response, survival, and side effects that were observed in NSCLC patients treated with the taxoid docetaxel can be summarized as follows: (a) patients with low AAG levels have a greater response rate to treatment with a taxoid than patients with high AAG levels; (b) patients with low AAG levels being treated with a taxoid survive longer than patients with high AAG levels; and (c) patients with low AAG levels will be more likely to experience adverse side effects from taxoid treatment relative to patients with high AAG levels.

As presented in the Examples below, the study shows that a patient having a low AAG level (≤1.11 g/L) had a response rate of 41.3% compared to a 15.9% response rate for patients with high (≥1.85 g/L) AAG levels. Accordingly, a patient's AAG level can be determined using the methods described above, preferably the Bienvenu et al. method, and the patient's observed AAG level may be compared to the population's predetermined AAG levels and the patient's AAG level classified into a low, intermediate, or high category. If the patient has a low AAG level, it can be predicted that the patient will have an increased chance of response to treatment. Similarly, if the patient's AAG level falls into the high AAG level category, it can be predicted that the patient will have a reduced chance of responding to treatment with a taxoid. Based on these predictions, treatment options may be considered, including, for example, maintaining the treatment at the current taxoid dosage, adjusting the taxoid dosage and/or expanding the treatment to include additional chemotherapeutic, surgical, or radiological treatment.

With regard to survival, patients having a low AAG level (≤1.11 g/L) had a median survival of 15.6 months. Patients having an intermediate AAG level (1.12 to 1.84 g/L) of AAG

had a median survival of 9.2 months and patients with a high level (≥1.85 g/L) of AAG had a median survival of 5.5 months. Accordingly, a patient's AAG level can be determined using the methods described above, preferably the Bienvenu et al. method and the patient's observed AAG level may be classified as having a low, intermediate, or high level of AAG compared to the population's predetermined AAG levels. Based on the patient's AAG level, the patient may be predicted to have a period of survival measured from the initiation of taxoid treatment to be of long, intermediate, or short duration. For example, a patient having a low AAG level would be expected to have a longer survival than a patient with intermediate or high levels or AAG. For patients with intermediate or high AAG levels, treatment options may be considered, including, for example, maintaining the treatment at the current taxoid dosage, adjusting the taxoid dosage and/or expanding the treatment to include additional chemotherapeutic, surgical, or radiological treatment.

With regard to side effects, as the AAG level varied from low (≤1.11 g/L) to high (≥1.85 g/L), there was approximately a 50% reduction in the odds of experiencing an adverse side effect (febrile neutropenia or infection or grade 3 diarrhea). Accordingly, a patient's AAG level can be determined using any of the methods described above, preferably the Bienvenu et al. method, and the patient's observed AAG level can then be classified as low, intermediate, or high AAG level compared to the population's predetermined AAG levels. If the patient's AAG level is in the low range, it can be predicted that the patient will have an elevated chance of experiencing side effects. If the patient is predicted to have an elevated chance of experiencing side effects, consideration can be given to lowering the patient's dose of the taxoid so as to reduce the chances of undesirable side effects. Reduction of the dosage of the taxoid must be balanced against reduction in the efficacy of treatment.

Examples

The following examples are representative of the practice of the invention.

Example 1

This example is illustrative of the present invention. It provides information stemming from a study of cancer patients who were treated with a taxoid (docetaxel) and concerning the relationship between AAG levels and a variety of physiological effects, including, for example, side effects. This study in its entirety is reported in applicant's U.S. provisional patent Application No. 60/114,520, filed December 30, 1998. The results of this study were published in Bruno et al., *Journal of Clinical Oncology*, Vol. 16, No. 1, p. 187-196 (1998).

The Patient Pool

Data were prospectively collected from patients entered in twenty-four Phase II open, non-randomized studies conducted from May 1992 to March 1994 to assess docetaxel clinical efficacy in a variety of tumor types including breast cancer, non small cell lung cancer, ovarian cancer, head and neck cancer, melanoma, renal cancer, colorectal cancer, gastric cancer, small cell lung cancer, and soft-tissue sarcoma. The studies were conducted in over 50 centers in Europe and three centers in the United States.

Criteria for eligibility included histology, at least one bidimensionally measurable lesion, adequate bone marrow reserve (absolute neutrophil count > 2,000/μL), adequate renal function (normal creatinine level) and liver function (total bilirubin level < 1.25 x ULN, SGOT (ALT) ≤2 x ULN or ≤3 x ULN in case of proven liver metastases). According to the tumor type, patients could have received various extents of prior treatment. The starting dose of docetaxel was either 75 mg/m² or 100 mg/m² given as a 1-hr infusion every 3 weeks. Dose reduction (25 %) or delay of subsequent courses were permitted, based on the degree of toxicity observed.

Most of the patients who registered (721/936, 77%) were sampled and among them, 81 were not considered evaluable for the study for the following reasons: not sampled at the first course (n=12, 1.7%); lack of documentation of samples (n=32, 4.4%); samples lost during transfer from the clinical sites to the analytical laboratory (n=18, 2.5%); or during assay procedure (n=19, 2.6%). Overall, 640 patients (89% of patients sampled, 68% of patients treated) were evaluable at first course.

Measurement of AAG Levels

AAG levels were determined by a variety of methods, primarily by the Bienvenu et al. laser nephelometry method. (See Bienvenu et al. Clinical Chemistry, 27(5), 721-726 (1981) and Example 3.)

Sampling Strategy

The aim of the sampling strategy in connection with taking blood samples from the patients was to define the full pharmacokinetic profile over the population, the so called "full screen" approach (Sheiner LB, Benet L Z: Premarketing observational studies of population pharmacokinetics of new drugs. Clin Pharmacol Ther 38: 481-487, 1985), by drawing a few samples per patient and varying (randomizing) the sampling times among patients (Hashimoto Y, Sheiner LB: Designs for population pharmacodynamics : Value of pharmacokinetic data and population analysis. J Pharmacokinet Biopharm 19: 333-353, 1991).

Recognizing the goal of individual estimates, the sampling strategy design was based on optimal individual sampling times computed using preliminary population PK parameter estimates obtained from Phase I data (Launay-Iliadis MC, Bruno R, Cosson V, et al: Population pharmacokinetics of docetaxel during Phase I studies using nonlinear mixed-effect modeling and nonparametric maximum-likelihood estimation. Cancer Chemother Pharmacol 37: 47-54, 1995). The sampling times were D-optimal (D'Argenio DZ: Optimal sampling times for pharmacokinetic experiments. J

Pharmacokinet Biopharm 9:739-756, 1981) and were computed using the APIS package, version 3.03a (Iliadis A, Brown AC, Huggins ML: APIS : A software for model identification, simulation and dosage regimen calculations in clinical pharmacokinetics. Comput Methods Programs Biomed 38: 227-239, 1992). Recognizing the goal of population estimates, separate sampling schedules, each consisting of early, mid and late time samples, were used to assure that the population PK samples were well spread across the available sampling time range.

There were 6 D-optimal sampling times (OST) for a three-compartment PK model (involving 6 parameters). OSTs were computed over a 0-24 hours observation interval. The estimated times (h:min) were: 0:30 (mid-infusion) or 1:00 (end of infusion), 1:15, 1:45, 3:45, 8:20 and 24:00.

The blood-sampling strategy consisted of four different sampling schedules (Table 1 below) which were assigned randomly to patients at study entry. Each schedule consists of 3 sampling times ranging between mid-infusion and 6 hours (5 hours post infusion). The first sample was always taken during the infusion, either mid infusion or just (5 minutes) before the end of the 1 hour-infusion. The two other samples were drawn within 5 hours after the end of infusion. Six hours was the maximum observation time in order to comply with outpatient status. However, when possible (e.g. for inpatients), one point could be replaced by a late sample drawn any time between 12 and 24 hours. A predrug sample (optional) was also requested to check the absence of analytical interference in patient plasma.

TABLE 1

SAMPLING STRATEGY IMPLEMENTED IN PHASE II STUDIES

Sampling Schedule No.	Sampling Times		
	1	2*	3
	During Infusion	After Infusion	
		Minutes	Hours
1	5 minutes before end	10	2
2	30 minutes after start	20	3
3	5 minutes before end	30	4
4	30 minutes after start	60	5

* When possible, this sample will be replaced by a blood sample obtained at a later time, i.e., any time between 12 and 24 hours post infusion.

A pharmacokinetic case report form (PK CRF) was designed to document actual sampling times as well as actual time of beginning and end of infusion. In some patients experiencing infusion-related hypersensitivity reactions, administration was interrupted and then resumed shortly after (e.g. 30 minutes). Actual times of starting and stopping the 2nd infusion were also documented on the PK CRF. Docetaxel was assayed in plasma samples using high performance liquid chromatography and UV detection after solid-phase extraction (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992) in 2 different cross-validated centers.

Pharmacokinetic Data Analysis

The collected data permitted elaboration and validation of a population pharmacokinetic model relating docetaxel clearance to patho-physiologic factors. This analysis has recently been reported (Bruno R., Vivier N., Vergniol J.C., et al: A population pharmacokinetic model for docetaxel (Taxotere®) : Model building and validation. J Pharmacokinet Biopharm 24:153-172, 1996). Population parameters from this analysis were used as prior information to estimate each

individual's pharmacokinetic parameters from his plasma concentrations using Bayesian estimation as implemented in the NONMEM computer program (version IV, level 2.0) (Beal S L, Boeckman AJ, Sheiner LB. NONMEM. User's Guide Part I to VI. University of California at San Francisco, San Francisco, 1988 - 1992).

The PK model was a three-compartment structural model with first-order elimination. The basic parameters were elimination clearance (CL, L/h), volume of distribution of the central compartment and intercompartmental rate constants. The inter-patient variability of PK parameters was modeled as (e.g. for CL):

$$CL_j = \hat{CL}_j \exp(\eta_{jCL})$$

where η_{jCL} denotes the (proportional) difference between the true parameter (CL_j) of individual j and the typical value in the population $\hat{CL}_j$ according to covariable values affecting $\hat{CL}$ for the j^{th} individual. Residual variability was modeled as proportional, consistent with the constant coefficient of variation of the assay measurement error (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992).

Individual plasma clearance (CL_j), area under the plasma concentration-time curve (AUC_j), peak plasma level, and time that plasma levels were greater than given threshold levels were used as measures of drug exposure.
CL_j was directly estimated by the Bayesian CL after fitting. Based on the estimate of CL_j, the following clearance factor (CLf) was generated:

$$CLf_j = (\text{mean CL}) / CL_j$$

Note that CLf_j is inversely proportional to CL_j : it takes values less than 1 for patients with clearance greater than the mean, and values greater than 1 for patients with clearance less than the mean (e.g. 2.0 for a 50 % decrease in clearance). Use of this derived parameter facilitates the

interpretation of pharmacokinetic/pharmacodynamic (PK/PD) models in term of clearance changes, as discussed below. AUC_j is computed as :

AUC_j ($\mu g \cdot h/mL$) = $Dose_j$ (mg) / CL_j (L/h)

Peak plasma level was taken to be the model predicted concentration at the end of infusion. Duration of exposure to plasma levels greater than 0.16 μg/mL (0.20 μM) ($t_{0.20}$), 0.080 μg/mL (0.10 μM) ($t_{0.10}$) and 0.040 μg/mL (0.05 μM) ($t_{0.05}$) was computed from estimated parameters using the implicit equation solver of EXCEL spread sheet, version 5 (Microsoft Corporation).

PK/PD analysis was conducted using as independent variables individual estimates, CLf_j, other exposure parameters (see above) and several other covariables related to the patient's patho-physiological status (demographics, disease spread) and extent of prior treatment. Docetaxel dose (mg/m^2), either given at first course or cumulative, was also considered as an independent variable measuring drug exposure.

Objective response rate, time to first response, and time to progression were selected as the efficacy endpoints (dependent variables). Only data from patients with breast cancer and non-small cell lung cancer (NSCLC) were analyzed. Assessment of tumor response was made every six weeks according to WHO criteria. Objective responses (complete responses (CR) and partial responses(PR)) were confirmed after a minimum of 4 weeks and were reviewed by an independent panel. Time to first response was calculated from the first docetaxel infusion up to the date of the first objective response either CR or PR whichever occurred earlier. Time to progression was calculated from the first docetaxel infusion up to the date of progression.

For safety, the following endpoints were considered among all tumor types:

- Neutropenia (NCI Grade) at first course.
- Febrile neutropenia at first course. Febrile neutropenia was defined as fever > 38°C (NCI grade ≥ II) with a

concomitant NCI grade 4 neutropenia (neutrophil count < 500/ μL) requiring antibiotics and/or hospitalization.

- Time to onset of fluid retention calculated from the first docetaxel infusion up to the date of the first sign and/or symptom of fluid retention (peripheral edema, pleural or pericardial effusions, ascites or weight gain).

Logistic regression was used to relate categorical endpoints, such as response rate and neutropenia grade, to the independent variables, while Cox regression was used for time to first response, time to progression and time to onset of fluid retention. Dose was the only time-dependent covariate in the Cox model. Model development involved stepwise inclusion and deletion of covariates. Significance levels for variable entry or removal at each step was $P < 0.10$; however, a final elimination pass, using $P < 0.05$ was used to determine the covariates kept in the final model. The median time to onset of fluid retention was estimated using the Kaplan-Meier method. Analyses were carried out using the SAS software (SAS version 6.11; SAS Institute Inc., Cary, NC).

Discussion of Results

Typical individual PK profiles are shown in Fig. 1 illustrating two of the four sampling schedules. The full population PK profile achieved by varying the sampling scheme across patients is illustrated in Figure 2 (data from a subset of 254 patients). This profile comprises 716 data points, that is, a mean of 2.8 per patient (range 1 to 5). Overall, a fair number of late samples was obtained (67 samples over 50 patients).

Patient characteristics at baseline

Patient characteristics are summarized in Table 2. Median age was 56 years, 42% were males and 58% females, 231 patients (36%) had breast cancer and 189 (30%) had NSCLC. Thirty-two percent of the patients were asymptomatic (WHO performance status of 0), whereas performance status of 1 and 2 were reported in 54% and 14% of the patients respectively. Thirty-three percent of patients had $\geq$ 3 organs involved, 82

% had visceral metastases, 35 % had liver metastases and 45% had previously been treated with chemotherapy. Most of the patients (95%) received 100 mg/m² as initial dose. Initially no premedication was used. Various premedication regimens (anti-H1 ± anti-H2 and/or corticosteroids either short term (≤ 2 days) or long term (≥ 3 days)) were subsequently given in some studies to prevent hypersensitivity reactions and fluid retention occurring during treatment. Few patients (n=25, 3.9%) received the five-day dexamethasone, presently recommended, premedication (8 mg orally twice daily starting the day before docetaxel administration).

TABLE 2

PATIENT CHARACTERISTICS AND DOCETAXEL EXPOSURE (N=640)

	COUNT		MEDIAN	5% TO 95% PERCENTILE
	NO.	%		
Age, years			56	38-71
Sex				
Male	270	42		
Female	370	58		
WHO performance status				
0	202	32		
1	342	54		
2	90	14		
Total protein (g/L)			71	59-81
Albumin (g/L)			41	31-48
AAG (g/L)			1.34	0.76-2.59
Elevated liver enzymes	26	4.1		
Tumor type				
Breast	231	36		
NSCLC	189	30		
Other	220	34		
Disease spread				
No. of disease sites ≥3	214	33		
Visceral involvement (yes)	522	82		
Liver metastasis (yes)	221	35		
Prior treatments				
Chemotherapy (yes)	289	45		
No. of prior regimens (≥2)	110	17		
Taxotere treatment/exposure				
Initial dose (mg/m²)				
75	31	5		
100	609	95		
CL (L/h)			36.3	17.5-59.3
CLf			1.02	0.622-2.11

	COUNT		MEDIAN	5% TO 95% PERCENTILE
	NO.	%		
AUC (μg-h/mL)			4.81	2.93-9.52
Peak (μg/mL)			3.26	1.93-5.76
$t_{0.20}$ (hours)			2.41	1.52-6.16 (0.858†)
$t_{0.10}$ (hours)			3.65	2.24-16.7 (0.856†)
$t_{0.05}$ (hours)			9.60	3.38-30.7 (0.838†)
Premedication				
None	252	39		
Recommended (5 days dexamethasone)	25	5		
Other	363	57		

* Patients with concomitant elevations of transaminases (>1.5 x ULN) and alkaline phosphatase (>2.5 x ULN).

† Correlation coefficient with AUC.

Individual PK parameter estimates

Individual estimates of PK and exposure parameters are given in Table 2. The continuous lines in Fig. 1 denote fits of patient data obtained using Bayesian estimation. In this large patient population, median clearance was 36.3 L/h which is a value very close to the value of 35.6 L/h previously estimated from Phase I data (Launay-Iliadis et al. Cancer Chemother Pharmacol 37:47-54 (1995)) and varied from 17.5 L/h to 59.3 L/h (5% to 95% percentile range). Representative exposure parameters were AUC: 4.81 μg·mL/h and peak: 3.26 μg/mL. Duration of exposure greater than threshold levels varied from 2.41 hours (0.20 μmol/L) to 9.60 hours (0.05 μmol/L). All of the measures of duration of exposure were strongly correlated with AUC r $\geq$ 0.838, Table 2).

Pharmacokinetics/Pharmacodynamics - Efficacy

No significant relationship was found between any estimate of docetaxel exposure and either objective response rate, time to first response or time to progression in breast cancer (201 evaluable patients, response rate: 56%). The number of disease sites was a significant predictor of response for all endpoints ($p<0.05$), baseline alpha-1-acid glycoprotein level (AAG) and number of prior chemotherapy

regimens were additional predictors ($p<0.005$) of time to progression.

Regarding NSCLC (151 evaluable patients, response rate : 29%), docetaxel AUC at first cycle was a significant predictor ($p=0.0232$) of time to progression after adjusting for other covariates (see Table 3). AUC was the only measure of docetaxel exposure to reach statistical significance. The median time to progression was 99 days (95% confidence interval: 84 - 121 days). According to this model, the risk of progression is decreased by 11 % per unit AUC and by 43 % for 5 AUC units (e.g. from the median to about the 95 percentile in this population). In addition, duration of exposure over 0.10 µmol/L was the only measure of exposure to reach borderline statistical significance ($p\sim0.10$) in predicting either response rate or time to first response. Of note, baseline AAG was a significant predictor of response for all endpoints ($P<0.005$).

TABLE 3

NSCLC: COX REGRESSION MODEL FOR TTP (N=151)

PREDICTOR	P	RISK RATIO	95% CI
Cumulative dose*	.0002	0.997	0.995-0.998
No. of disease sites	.0011	1.293	1.109-1.507
AAG	.0022	1.757	1.225-2.518
Performance status	.0177	1.483	1.071-2.055
AUC	.0232	0.891	0.807-0.984

Note: Progression occurred in 84% of patients (127 of 151).
Abbreviation: CI, confidence interval.

* Time-dependent covariate.

Neutropenia

Neutropenia was analyzed at first course in 582 patients. Most of the patients (375/582, 64%) experienced grade 4 neutropenia. Several strong predictors of grade 4

neutropenia were identified including the various measures of docetaxel exposure with Clf, AUC and $t_{0.20}$ having the strongest effects (P<0.0001). After adjustment for the other covariates in the model, dose no longer had a significant effect. Clearance factor, CLf was retained in the final model (Table 4 below) since it greatly facilitates the interpretation of the model in terms of clearance change. The incidence of neutropenia grade 4 was related to the baseline neutrophil count (P=0.0002) and the number of previous regimens (P=0.0002) as expected. Baseline AAG level and first course exposure were the most significant predictors (P<0.0001). The higher the AAG level at baseline, the lower the odds of experiencing grade 4 neutropenia during the first course of treatment. According to the logistic regression model, a 1-g/l increase of baseline AAG (for example, from the median to about the 95 percentile in this population) results in a 83% decrease in the odds of experiencing grade 4 neutropenia. The effect of drug exposure change is the opposite with a 430% (4.3 fold) increase of the odds of grade 4 for a 1 unit increase in CLf. A 1 unit increase in CLf corresponds to a 50% decrease of clearance which is also a change from the median to the 95th percentile in this population).

TABLE 4

LOGISTIC REGRESSION MODEL FOR GRADE 4 NEUTROPENIA (N=582)

PREDICTOR	P	ODDS RATIO	95% CI
AAG	<.0001	0.17	0.10-0.29
Clf	<.0001	4.26	2.46-7.39
Baseline count	.0002	0.84	0.77-0.92
No. of previous regimens	.0002	1.72	1.30-2.29

Note: Incidence, 64% (375 of 582 patients)

Febrile neutropenia was observed in 26 of the 582 patients (4.5%) at first cycle. The model for this endpoint

was similar to that for neutropenia grade 4 with exposure (CLf) and AAG being the only significant predictors (Table 5 below). In this model, change of exposure due to a 50% decrement in clearance would result in a 300% (3.0 fold) increase in the odds of febrile neutropenia. The model-predicted probability of febrile neutropenia as a function of Clf (AAG fixed at the median) is illustrated in Figure 3.

TABLE 5

LOGISTIC REGRESSION MODEL FOR FEBRILE NEUTROPENIA (N=582)

PREDICTOR	P	ODDS RATIO	95% CI
Clf	.0012	3.03	1.55-5.93
AAG	.0056	0.28	0.12-0.69

Note: Incidence, 4.7% (26 of 582 patients).

Fluid retention

Fluid retention occurred in 53% of 631 evaluable patients. The median time to onset was 85 days (95% confidence interval: 81 to 92 days). Patients with breast and ovary carcinoma had disease related baseline symptoms resulting in a higher baseline risk than patients with other tumor types. The analysis was stratified, therefore, by tumor type with breast and ovary combined and other tumor types combined. Fluid retention incidence was 73% (172 of 236) in patients with breast or ovary tumors and 41% (163 of 395) in patients with other tumor types. Of note, few patients (n=25, 4%) received the presently recommended 5-day dexamethasone premedication in this population since this premedication was only recommended after the majority of these patients had been treated.

Owing to the cumulative nature of docetaxel induced fluid retention, dose was treated as a time-dependent covariate in the analysis. Cumulative dose was the most important predictor in the final Cox regression model (Table 6 below). However, several other baseline covariates had independent predictive power including AAG and total protein

levels. Drug exposure at first course was also highly significant in predicting the time to onset of fluid retention, after adjustment for the effect of cumulative dose. The duration parameter, $t_{0.20}$ was the most significant (P=0.0029) measure of exposure for this regression.

TABLE 6

COX REGRESSION MODEL FOR TIME TO ONSET OF FLUID RETENTION

(N=631)

PREDICTOR	P	RISK RATIO	95% CI
Cumulative dose*	<.0001	1.005	1.003-1.007
$t_{0.20}$	.0029	1.087	1.029-1.148
Total protein	.021	0.980	0.964-0.997
AAG	.014	0.746	0.591-0.942

Note: Incidence, 53% (335 of 631 patients).

Stratification: breast/ovary-236 patients/incidence, 73%; other-395 patients/incidence, 41%.

*Time-dependent covariate.

According to the model, the risk of experiencing fluid retention at any time is increased by 64% for each additional cycle at 100 mg/m². An increase of $t_{0.20}$ by 4 hours i.e. roughly from the median (2.41 hours) to the 95th percentile (6.16 hours) at first course increases the risk by 40% beyond the effect of cumulative dose.

Baseline AAG level was a significant predictor of all the PD endpoints investigated in this study.

Example 2

This example is illustrative of the present invention. It provides information stemming from a study of alpha-1-acid glycoprotein as an independent predictor of response and survival in patients with non-small cell lung cancer treated with docetaxel.

The Patient Pool

The data for this study was prospectively collected from unresectable and metastatic NSCLC patients entered into six Phase II open label, non-randomized studies of docetaxel (Burris H, Eckardt J, Fields S, et al: Phase II trials of Taxotere in patients with non small cell lung cancer. Proc Am Soc Clin Oncol 12: 335, 1993 (abstr 1116); Cerny T, Kaplan S, Pavlidis N, et al: Docetaxel (Taxotere) is active in non-small-cell lung cancer: A phase II trial of the EORTC Early Clinical Trials Group. Br J Cancer 70: 384-387, 1994; Fossella FV, Lee JS, Murphy WK et al: Phase II trial of docetaxel for recurrent or metastatic non-small cell lung cancer. J Clin Oncol 12: 1238-1244, 1994; Francis PA, Rigas JR, Kris MG et al: Phase II trial of docetaxel in patients with Stage III and IV non-small cell lung cancer. J Clin Oncol 12: 1232-1237, 1994; Fossella FV, Lee JS, Shin DM, et al: Phase II study of docetaxel for advanced or metastatic platinum-refractory non-small-cell lung cancer. J Clin Oncol 13: 645-651, 1995; Miller VA, Rigas JR, Francis PA, et al: Phase II trial of a 75 mg/m^2 dose of docetaxel with prednisone premedication for patients with advanced non-small cell lung cancer. Cancer 75: 968-972, 1995). Detailed information and clinical trial results for these studies have been previously reported.

The criteria for eligibility included confirmation of non-small cell lung cancer, one or more bidimensionally measurable lesion, adequate bone marrow (absolute neutrophil count > 2,000/mL), renal (normal creatinine) and hepatic function (total bilirubin < 1.25 x upper limit of normal (ULN), alanine aminotransaminase (ALT) ≤ 2 x ULN). According to the study design, patients may have received prior treatment. The initial docetaxel dose for most patients was 100 mg/m^2 given as a 1-hr infusion every 3 weeks. Dose reduction of twenty-five percent or delay of subsequent courses of therapy was permitted, based on the grade of toxicity observed. These studies were part of the 22 Phase II studies reported in a previous PK/PD analysis of docetaxel (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics

Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998).

Measurement of AAG Levels

AAG levels were determined by a variety of methods, primarily by the Bienvenu et al. laser nephelometry method. (See Bienvenu et al. Clinical Chemistry, 27(5), 721-726 (1981) and Example 3.)

Pharmacokinetic Data

Pharmacokinetic assessment was performed at the first cycle of treatment. The design of the sampling strategy was presented in Example 1 and in detail in Bruno *et al* (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics /Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998). Briefly, the sampling strategy consisted of four different sampling schedules of 3 sampling times which were randomly assigned to patients upon study entry. Docetaxel was assayed in plasma samples using high performance liquid chromatography and UV detection after solid-phase extraction (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992).

From the population pharmacokinetic parameters (Bruno R, Vivier N, Vergniol JC et al: A population pharmacokinetic model for docetaxel (Taxotere®) : Model building and validation. J Pharmacokinet Biopharm 24:153-172, 1996), Bayesian methods were used to estimate each individual's pharmacokinetic parameters from the patient's plasma concentrations (Baille P, Bruno R, Schellens JHM et al: Optimal sampling strategies for Bayesian estimation of docetaxel (Taxotere®) clearance. Clin Cancer Res 3:1535-1538, 1997). The NONMEM computer program was employed for these studies (version IV, level 2.0) (Beal SL, Boeckman AJ, Sheiner LB. NONMEM. User's Guide Part I to VI. University of

California at San Francisco, San Francisco, 1988 - 1992). The PK model used a three-compartment structural model with first-order elimination and the PK parameters considered for this analysis are CL, and AUC.

Clinical Endpoints

The following clinical endpoints were considered for this analysis.

Safety : Febrile neutropenia, infections, grade 3/4 stomatitis, grade 3/4 diarrhea and severe asthenia, reported during the first course of therapy were considered as safety endpoints. These parameters were selected as they typically require dose reduction or treatment delay. Stomatitis and diarrhea were defined and graded using the Common Toxicity Criteria of U.S. National Cancer Institute whereas COSTART classification was used for asthenia. Febrile neutropenia was defined as body temperature > 38°C with concomitant NCI grade 4 neutropenia (neutrophil count < 500/mL) requiring antibiotics and/or hospitalization.

Due to the small number of patients and low incidence of severe adverse events, these safety endpoints were pooled for analysis.

Response rate : The patients were considered to be a responder when they experienced either a partial response (PR) or a complete response (CR) using standard criteria. Patients with minor responses (< 50 % reduction in tumor size), evaluable disease, stable disease and patients with disease progression were considered as non responders. Responses had to be confirmed after a minimum of 4 weeks and were reviewed by an independent panel.

Survival : Survival was calculated from the date of the first infusion to the date of death, last contact for patients lost of follow-up, or a cut-off date for patients alive at the time of closure of the data set.

Data Analysis

Three categories of independent variables thought to affect survival in NSCLC were considered for this analysis. Firstly, docetaxel exposure as assessed by the cumulative dose, or CL and AUC at first course Secondly, the patient characteristics including age, gender, performance status, alpha-1-acid glycoprotein, lactate dehydrogenase, baseline neutrophil count, time from initial diagnosis of NSCLC, number of disease sites, visceral cancer involvement, hepatic metastasis and bone metastasis. Thirdly, the extent of prior treatment reported as prior chemotherapy, number of prior chemotherapy regimens, prior cisplatin, and prior radiotherapy.

A logistic regression was used to relate binary endpoints, such as the incidence of severe adverse events and response rate, to the independent variables, while a Cox regression was used for the survival analysis. Cumulative docetaxel dose was the only time-dependent covariate used in the Cox model. Univariate and multivariate analyses were conducted. The multivariate model involved a stepwise selection of covariates starting from the null model. Significance levels for variable entry or removal at each step in the development of the multivariate model were $p<0.10$ and $p<0.05$, respectively. The median survival was estimated using the Kaplan-Meier method. Analyses were carried out using the SAS software (SAS version 6.12; SAS Institute Inc., Cary, NC).

Discussion of Results

Patient characteristics at baseline

Overall, 189 patients of the 269 NSCLC patients entered in the six Phase II studies of docetaxel (70 %) had pharmacokinetic data available for analysis. Nine patients received 75 mg/m^2 of docetaxel as their initial dose, and all other patients (n=180) received 100 mg/m^2. This analysis was restricted to the patients treated with 100 mg/m^2 of

docetaxel. Among these patients, 143 were evaluable for response, however, the analysis was conducted on the intent-to-treat population of 189 patients. Some models were reassessed on the evaluable patient population as a sensitivity analysis to examine their affects on the predictor outcomes. The patient characteristics are summarized in **Table 7.** Median age for this population of NSCLC patients was 61 years, two thirds were male, 82% of the patients had a WHO performance status of 0 to 1. Most of the patients were chemotherapy naive (71%), and had metastatic disease (77%).

Table 7. Patient characteristics and docetaxel exposure (n = 180)

percentile		Number	%	median	5%-95%
Age (years)				61	43-72
Sex	Male			118 (66)	
	Female			62 (34)	
WHO performance status		0		35 (19)	
		1		113 (63)	
		2		32 (18)	
α1-acid glycoprotein (g/l)				1.42	0.84-2.71
Time from diagnosis (month)				4.7	0.6-39
	>12 month			48 (27)	
Extent of disease					
Number of disease sites		1		48 (27)	
		2		70 (39)	
		3		42 (23)	
		≥4		20 (11)	
Liver metastasis				34 (19)	
Prior treatments					
Chemotherapy				52 (29)	
Number or prior regimen		0		128 (71)	
		1		34 (19)	
		≥2		18 (10)	

percentile	Number % median	5%-95%
Prior platinum	43 (24)	
Radiotherapy	70 (39)	
<u>Docetaxel exposure</u>		
CL (L/h)	35.7	17.8-58.8
AUC (mg.h/mL)	4.98	3.24-9.76

Individual PK parameter estimates

Individual estimates of PK and exposure parameters are given in **Table 7.** In this NSCLC patient population, the median clearance was 35.7 L/h varying from 17.8 L/h to 58.8 L/h (5% to 95% percentile range). This clearance distribution was very similar to that of the larger population of patients with various tumor types with a median of 36.3 L/h (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics /Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998); The observed median AUC was 4.98 mg·mL/h with a 5% to 95% percentile range 3.24 mg·mL/h to 9.76 mg·mL/h.

Severe adverse events

Twenty-five patients (13.9 %) experienced at least one severe adverse event during the (TOX) first cycle of therapy (**Table 8**). Docetaxel exposure as measured by the AUC was the only significant predictor of these adverse events ($p<0.0001$). A high AUC was associated with increased probability of experiencing any of the severe toxicities. Age of the patients had a borderline significant effect ($p = 0.056$), with older patients showing a trend towards a higher probability of experience a severe adverse event.

Table 8. Incidence of adverse events at cycle 1

	number	%
Febrile Neutropenia	7	3.9
Infection	8	4.4
Stomatitis (Grade 3, 4)	3	1.7
Diarrhea (Grade 3, 4)	10	5.6
Asthenia (severe)	2	1.1
Endpoint		
TOX*	25	13.9
TOX1**	23	12.8

*patients experienced at least one event

**patients experienced febrile neutropenia or infection or grade 3 diarrhea

Subsets of associated toxicities were also analyzed for their correlative significance. In all subsets, AUC was the only significant predictor of these severe adverse events. In one subset that included febrile neutropenia or infection or diarrhea (TOX1), with 23 adverse events, (12.8%), AAG reached borderline significance (p=0.0505) in addition to AUC.

The odds ratio for the logistic regression models calculated for the relevant covariate changes from the 25th to the 75th percentiles are given in **Table 9**. According to the logistic model, the odds of experiencing a severe adverse reaction was approximately 2 fold greater for a change in AUC from 4.2 to 6.5 mg·h/mL. While, an increase in the AAG from 1.11 to 1.85 g/L resulted in roughly a 50 % reduction in the odds of experiencing one toxicity event from the TOX1 group.

Table 9. Logistic regression models for adverse events at cycle 1

Endpoint	Predictor	p	Odds Ratio*	(95 % CI)
TOX	AUC (4.2 to 6.5 mg.h/mL)	0.0021	1.81	(1.24 - 2.64)
TOX1	AUC (4.2 to 6.5 mg.h/mL)	0.0005	2.37	(1.46 - 3.87)
	AAG (1.11 to 1.85 g/L)	0.0505	0.47	(0.22 - 1.00)

*odds ratio for covariate change from 25th to 75th percentiles

for AUC and for AAG

Response rate

The overall response rate was 29% in both intent-to-treat and evaluable populations. Baseline AAG was the only significant predictor of response rate (p=0.0039) with an odds ratio of 0.44 for a change in AAG from 1.11 to 1.85 g/L. An increase in the baseline AAG level was associated with a 56% decrease in the odds of response (**Table 10**). The response rate was 41.3% (95% CI : 27.0% - 56.8%) for patients with a low AAG (AAG $\leq$ 1.11 g/L, n = 46) and 15.9% (95% CI : 6.7% - 30.1%) for patients with a high AAG (AAG $\geq$ 1.85 g/L, n = 44).

Table 10. logistic regression model for response*

Predictor	p	Odds Ratio** (95 % CI)
AAG (1.11 to 1.85 g/L)	0.0039	0.44 (0.25 - 0.77)

* intent-to-treat population, response rate = 25.0 %

*odds ratio for AAG change from 25th to 75th percentiles

In the univariate analyses, in addition to baseline AAG levels, trends were observed for a lower odds of response in patients with metastatic disease (p=0.054), in patients who received radiotherapy prior to docetaxel treatment (p=0.055), in younger patients (p=0.080) and in patients with a poor performance status (p=0.080). However, when baseline AAG was included in the multivariate analysis, none of these covariates entered the model even at a significance level of p<0.10. Similar findings were very obtained for the patients with evaluable disease.

Survival

The most significant univariate predictors of survival were cumulative dose, baseline AAG and number of sites of disease (p<0.0001). Clearance or AUC, prior radiotherapy, gender and performance status were also significant predictors of survival (p<0.05). The risk of death decreased as the cumulative dose of docetaxel increased. However, an increased risk of death was observed for patients with higher AAG, two or more sites of disease, low CL or high AUC, poor performance status, female gender and for patients having received prior therapy.

Only cumulative dose, AAG and two or more disease sites remained significant in the multivariate analysis (**Table 11**). The risk of death decreased by 20 % for each additional cycle of treatment and roughly doubled in patients with a high AAG (1.85 g/L) compared to patients with a low AAG (1.11 g/L) and in patients with two or more sites of disease.

Table 11. Cox regression model for survival*

Predictor	p	Risk Ratio** (95 % CI)
Cumulative dose***(100mg/m²)	< 0.0001	0.82 (0.74 - 0.90)
AAG (1.11 to 1.85 g/L)	< 0.0001	1.76 (1.40 - 2.21)
No disease sites (< 2 to ≥ 2)	0.0049	1.96 (1.23 - 3.12)

* death occurred in 70.5 % of the patients (127 of 180 patients)

** risk ratio for change of covariates given in brackets

*** time-dependent covariate

When baseline AAG was not considered in the stepwise multivariate analysis, it was replaced by performance status (p=0.0053), gender (p=0.025) and prior radiotherapy (p=0.045). Therefore, the pretreatment AAG level appeared to be a more important predictor of survival in NSCLC patients treated with docetaxel than several other known prognostic factors. The median survival (**Table 12** and **Figure 4**) varied from 15.6 months in low AAG patients (AAG ≤ 1.11 g/L, n=46) to 5.5 months in high AAG patients (AAG ≥ 1.85 g/L, n=44). Patients with intermediate AAG values (n=90) had a median survival time of 9.2 months.

Table 12. Survival as a function of alpha-1-acid glycoprotein baseline level

	alpha-1-acid glycoprotein (g/L)			
	≤ 1.11* (n=46)	1.12 - 1.84 (n=90)	≥ 1.85** (n=44)	Log-Rank
median (month)	15.6	9.2	5.5	< 0.0001
95% CI	(11.8-20.0)	(6.4-11.4)	(4.1-7.5)	

* 25% quantile of AAG distribution

** 75% quantile of AAG distribution

Over the last decade performance status has been recognized as the most important predictor of response, and survival in patients with advanced NSCLC (Ginsberg RJ, Vokes EE, Raben A: Non-small cell lung cancer, in De Vita VT, Hellman S, Rosenberg SA (eds): Cancer Principles & Practice of Oncology. Volume 1, Chapter 30, Section 2, 5th Edition, Philadelphia, New York, Lippincott-Raven, 1997, pp 858-911). This study shows that NSCLC patients with a high baseline AAG have a lower response rate (14% compared to 44% in patients with a low AAG) and a markedly shorter survival (median of 5.5 month compared to 15.6 months in patients with a low AAG).

Example 3

This example is representative of methods used to determine AAG levels. 500 µL samples of blood were collected in a polystyrene microtube without anticoagulant by venous puncture. The serum was removed after centrifugation.

For laser nephelometry a Behring Laser Nephelometer module I was used. (Behringwerke, D-3550 Marburg/Lahn, Germany). Samples, standards, and antisera were diluted with sterile isotonic saline solution and 100 µL of 101-fold diluted sample were mixed in a microcuvette with 200 µL of a fivefold diluted anti-orosomucoid antiserum (LN serum anti-orosomucoid (AAG) SAW; Behringwerke). The cuvettes were shaken briefly and allowed to stand for 1 hour at room temperature, and the light scattered by the resulting antigen-antibody complexes was measured (in volts) with the nephelometer. A calibration curve was prepared by use of an 800mg/L standard solution of orosomucoid, diluted to give concentrations of 40, 20, 10, 5, 2.5, and 1.25 mg/L. The blank values (i.e., the light scattered by the empty cuvettes) were negligible (80-150 mV).

Example 4 - This example presents a clinical trial simulation for exploring the safety profile of docetaxel (Taxotere®) in cancer patients.

Docetaxel exposure and alpha-1-acid glycoprotein level (AAG) predict hematological toxicities of docetaxel (Bruno et al., *J. Clin. Oncol.*, 16, 187 (1998)). To assess the impact of increasing doses on the safety profile of docetaxel, in patients with different AAG levels, 100 complete trials were stochastically simulated (ACSL Biomed). In each trial, 600 patients were randomly assigned to groups of either low (L) (≤ 1.11 g/L), intermediate (I) (1.12-1.84 g/L) or high (H) (≥ 1.85 g/L) AAG and received 60, 75, 100 and 125 mg/m^2 of docetaxel intravenously over 1 hour. The simulated median AUC, median incidence of grade 4 neutropenia (GR4) and febrile neutropenia (FEB) were:

Group	Dose (mg/m^2)	60	75	100	125
	AUC (µg.h/mL)	2.7	3.4	4.5	5.6
	GR4 (%)	68.9	73.4	79.9	84.5
L	FEB (%)	4.3	5.3	7.3	10.1

Group	Dose (mg/m^2)	60	75	100	125
	AUC ($\mu g.h/mL$)	3.0	3.8	5.0	6.3
	GR4 (%)	41.1	46.5	56.6	66.3
I	FEB (%)	2.3	2.9	4.4	6.5
	AUC ($\mu g.h/mL$)	3.8	4.7	6.3	7.9
	GR4 (%)	13.3	17.5	26.1	35.7
H	FEB (%)	1.0	1.0	1.9	3.6

The results demonstrate that the dose response of docetaxel is markedly influenced by AAG and these results provide insights for the design of future trials.

Example 5 - This examples summarizes studies on Alpha-1-acid glycoprotein as an independent predictor of efficacy and survival in NSCLC patients treated with docetaxel (Taxotere®).

Baseline alpha-1-acid glycoprotein (AAG) and docetaxel docetaxel clearance (and/or exposure) were previously found to be independent predictors of docetaxel safety (all tumor types combined) and of time to progression (TTP) in NSCLC (Bruno et al., *J. Clin. Oncol.* (Vol. 16, No. 1, 1998, pp. 187-196)). The predictors of treatment outcome and survival of advanced NSCLC patients entered in 4 Phase II studies (n=180) of docetaxel (100 mg/m^2) were investigated using logistic and Cox multivariate regressions. Univariate analysis showed that compared to patients with high AAG ($\geq$ 1.92 g/L (75 percentile)), patients with low AAG ($\leq$ 1.09 g/L (25 percentile)) experienced more side effects (e.g. febrile neutropenia: 19% vs. 2.3%, p=0.02) but had a higher response rate (44% vs. 14%, p=0.002), a longer TTP (18 vs. 9.7 weeks, p=0.006) and a much longer survival: 16 months compared to 5.2 months ($p<0.0001$). In multivariate models, in addition to TTP (Bruno et al., *supra*), AAG was an independent prognostic factor for the incidence of severe side effects at first cycle (p=0.006 with an interaction with clearance), for response rate (odds ratio for non response in high AAG

patients: 5.5, p=0.006), and for survival (p<0.0001). In conclusion, low AAG is independently associated with better efficacy and longer survival in advanced NSCLC treated with docetaxel.

CLAIMS

1. A method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein comprising: (A) observing the patient's level of alpha-1-acid glycoprotein; (B) evaluating said level to determine the dosage of the taxoid to administer to the patient by comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said evaluation, recommending the dosage of the taxoid to administer to the patient.

2. The method of claim 1 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

3. The method of claim 1 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

4. The method of claim 3 wherein said cancer is non-small cell lung cancer.

5. The method of claim 1 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

6. A method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid comprising: (A) observing the patient's alpha-1-acid glycoprotein level; (B) comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a

population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said comparison, assessing the effect of continued treatment of the patient with respect to the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient.

7. The method of claim 6 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

8. The method of claim 6 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

9. The method of claim 8 wherein said cancer is non-small cell lung cancer.

10. The method of claim 6 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

11. The method of claim 6 wherein said patient is being treated with a dosage of about 55 to about 200 mg/m^2 of taxoid.

12. The method of claim 6 wherein said patient is being treated with about 55 to about 125 mg/m^2 docetaxel.

13. The method of claim 6 wherein said patient is being treated with about 135 to about 175 mg/m^2 paclitaxel.

14. A method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid comprising: (A) observing the patient's alpha-1-acid glycoprotein (AAG) level; (B) comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said comparison recommending the dosage of said taxoid to administer to said patient to reduce the incidence or severity of side effects that the patient may experience during treatment with said taxoid.

15. The method of claim 14 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

16. The method of claim 14 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

17. The method of claim 16 wherein said cancer is non-small cell lung cancer.

18. The method of claim 14 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

19. The method of claim 14 wherein said population of patients is being treated with a dosage of about 55 to about 200 mg/m^2 of said taxoid.

20. The method of claim 14 wherein said patient is being treated with about 55 to about 125 mg/m^2 of docetaxel.

21. The method of claim 14 wherein said patient is being treated with about 135 to about 175 mg/m^2 paclitaxel.

22. The method of claim 14 wherein the side effects are selected from the group consisting of neutropenia, infection, diarrhea, infusion-related hypersensitivity reactions, allopenia, neurotoxicity, mucositis, stomatitis, severe asthenia, fluid retention and myalgias.

23. The method of claim 22 wherein said side effect is neutropenia.

24. The method of claim 23 wherein said neutropenia is febrile neutropenia.

25. The method of claim 14 wherein said taxoid is docetaxel and said dosage is recommended to be less than about 100 mg/m^2.

26. The method of claim 14 wherein said taxoid is paclitaxel and said dosage is recommended to be less than about 175 mg/m^2.

27. The method of claim 14 wherein the recommended dosage is about 5 to about 35% below said common dosage.

28. The method of claim 14 wherein the recommended dosage is reduced by about 10 to about 30% below said common dosage.

29. The method of claim 14 wherein the recommended dosage is reduced to about 15 to about 27% below said common dosage.

PREDICTIVE METHODS

BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS

Abstract of the Invention

A method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein comprising observing the patient's level of alpha-1-acid glycoprotein, evaluating said level to determine the dosage of the taxoid to administer to the patient by comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said evaluation, recommending the dosage of the taxoid to administer to the patient. Also, a method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid comprising observing the patient's alpha-1-acid glycoprotein level, comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison, assessing the effect of continued treatment of the patient with respect to the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient. Also, a method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid comprising observing the patient's alpha-1-acid glycoprotein (AAG) level, comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison recommending the dosage of said taxoid to administer to said patient to reduce the incidence or severity of side effects that the patient may experience during treatment with said taxoid.

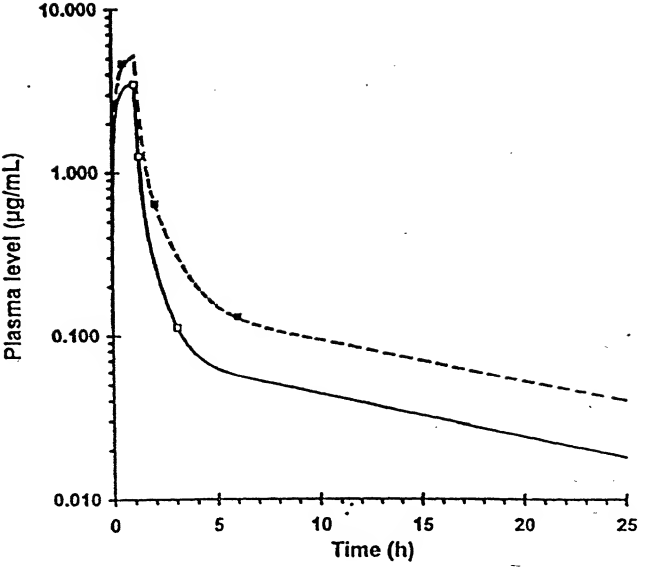

Fig 1. Docetaxel PK profile in representative patient with normal liver function (□) and patient with elevated hepatic enzymes (---■---). Lines denote model predictions after Bayesian estimation.

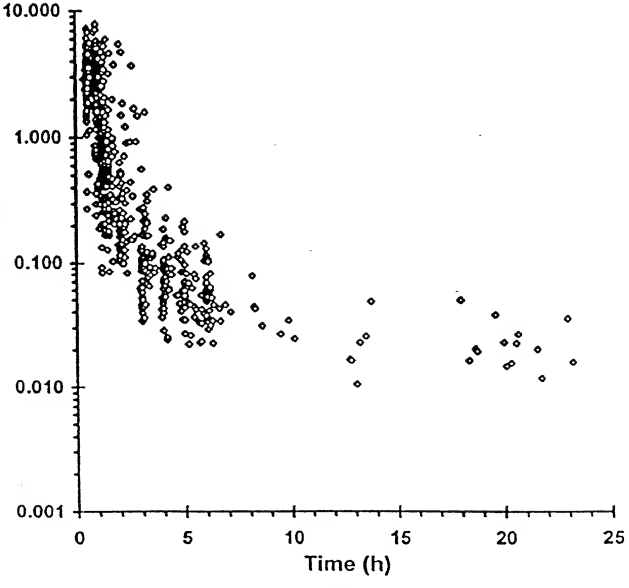

Fig 2. Docetaxel population PK profile in a subset of 254 patients.

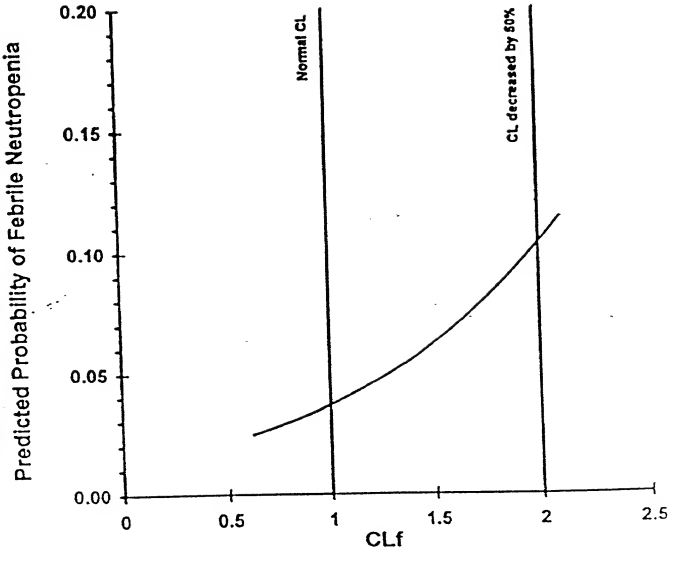

Fig 3. Model-predicted probability of febrile neutropenia as a function of CLf for a patient with median AAG. Reference vertical lines denote normal CL (CLf = 1) and 50% reduced CL (CLf = 2).

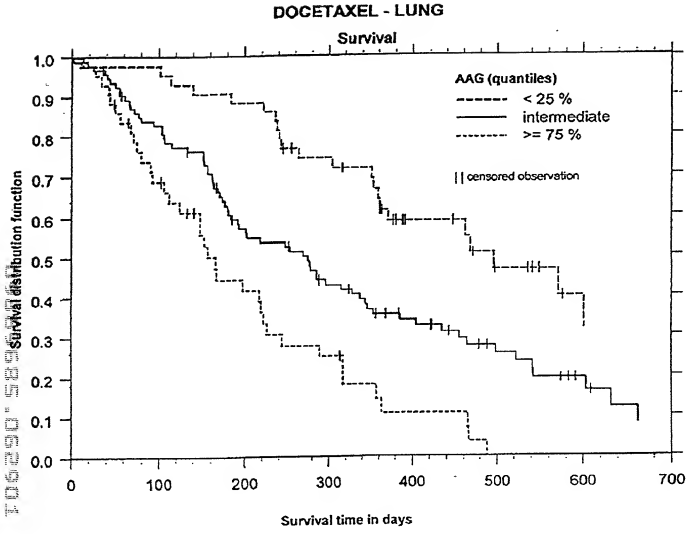

Figure 4 : survival curves in NSCLC patients with low (≤ 1.11 g/L, —), intermediate (1.12 to 1.84 g/L, ----) and high (≥ 1.85, -•-•-) baseline AAG (/censored observation)

Docket No.
P23,565-A USA

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **30 December 1999** as United States Application No. or PCT International Application Number _____

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
PCT/US99/31284	**PCT**	**30 December 1999**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional

60/114,520	30 December 1998
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US99/31284	30 December 1999	pending
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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United States Patent & Trademark Office

Office of Initial Patent Examination

Application papers not suitable for publication

SN 09869685 Mail Date 06/29/01

- ☐ Non-English Specification
- ☒ Specification contains drawing(s) on page(s)_____ or table(s) 1
- ☐ Landscape orientation of text ☐ Specification ☐ Claims ☐ Abstract
- ☐ Handwritten ☐ Specification ☐ Claims ☐ Abstract
- ☐ More than one column ☐ Specification ☐ Claims ☐ Abstract
- ☐ Improper line spacing ☐ Specification ☐ Claims ☐ Abstract
- ☐ Claims not on separate page(s)
- ☐ Abstract not on separate page(s)
- ☐ Improper paper size -- Must be either A4 (21 cm x 29.7 cm) or 8-1/2"x 11"
  - ☐ Specification page(s)_____ ☐ Abstract
  - ☐ Drawing page(s)_____ ☐ Claim(s)
- ☐ Improper margins
  - ☐ Specification page(s)_____ ☐ Abstract
  - ☐ Drawing page(s)_____ ☐ Claim(s)
- ☐ Not reproducible Section
  - Reason
  - ☐ Paper too thin ☐ Specification page(s)_____
  - ☐ Glossy pages ☐ Drawing page(s)_____
  - ☐ Non-white background ☐ Abstract
  - ☐ Claim(s)
- ☐ Drawing objection(s)
  - ☐ Missing lead lines, drawing(s)_____
  - ☐ Line quality is too light, drawing(s)_____
  - ☐ More than 1 drawing and not numbered correctly
  - ☐ Non-English text, drawing(s)_____
  - ☐ Excessive text, drawing(s)_____
  - ☐ Photographs capable of illustration, drawing(s)_____